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**Identification and Quantification of Thujone In a Case of Poisoning Due to Repeated Ingestion of an Infusion of *Artemisia vulgaris* L.**

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## Abstract

Plants of the *Artemisia* genus are used worldwide as ingredients of botanical preparations. This paper describes the case of a 49-year-old man admitted to the emergency room at a Zurich hospital in a manic state after the ingestion of 1 L of an infusion of *Artemisia vulgaris*. Two monoterpenic ketones,  $\alpha$ - and  $\beta$ -thujone, are present in various concentrations in *Artemisia spp*, but adverse effects have previously been associated only with essential oil from *Artemisia absinthium* and attributed to the inhibition of gamma-aminobutyric acid receptors, with consequent excitation and convulsions.

The aim of this work was to examine and quantify the possible presence of thujone in the patient's serum and urine. A High Performance Liquid Chromatography (HPLC) method with isocratic separation and fluorescence detection (FLD) was set up and validated. Serum thujone concentrations were found to be  $27.7 \pm 3.48$   $\mu\text{g/mL}$  at day 0 and  $24.1 \pm 0.15$   $\mu\text{g/mL}$  on day 1. Results were confirmed by a gas chromatography with flame ionization detection (FID). Poisoning due to thujone was thus confirmed, suggesting four possible scenarios: 1) an unusually high concentration of thujone in the *Artemisia vulgaris* ingested; 2) chronic exposure as the cause of the poisoning; 3) low metabolic efficiency of the patient; 4) contamination or adulteration of the plant material with other *Artemisia* species, e.g. *Artemisia absinthium*.

## Practical application

These results could aid research in the field of adverse effects of botanicals, lead to better understanding and management of similar cases of poisoning, and promote more informed use of natural products.

Word count: 3705

**Keywords:** thujone poisoning, *Artemisia* spp., adulteration, biomarkers, HPLC-FLD, gas-liquid chromatography, flame ionization detector

## Introduction

*Artemisia vulgaris* L. (mugwort) is a weed in the family of *Asteraceae*, widely distributed in Europe, Asia and North America. Its traditional use is mainly based on infusions with supposed antihypertensive, antispasmodic, anti-inflammatory and anthelmintic properties (Miller, 2000). Other applications have been suggested in the field of gynecology (dysmenorrhea and problems during labor) (Chevallier, 1996; Lee et al. 1998). *Artemisia vulgaris* was used as a flavor in beers before hops, and infusions of the leaves and flowering tops have been prescribed for digestive problems (Barney and Di Tommaso, 2003; Miller, 2000). The genus *Artemisia* is highly variable in morphology and phytochemical composition – approximately 60 different compounds have been identified in it (Abad, Bedoja, Apaza, and Bermejo, 2012). Thujone is a monoterpenic ketone naturally present in two stereoisomeric forms: (-)-3-isothujone (CAS 546-80-5) or  $\alpha$ -thujone and (+)-3-isothujone (CAS 471-15-8) or  $\beta$ -thujone (O'Neil, 2013). Figure 1 shows their chemical structures.

Thujone occurs in different quantities in *Artemisia* species. Its toxicological potential was emphasized by EMA (European Medicines Agency) in its monograph on both *Artemisia absinthium* L. herba (EMA, 2009a) and *Salvia officinalis* L. folium: “Thujone is reported to be neurotoxic and chemotypes with low content of thujone should be preferred. The intake of thujone should not exceed 3.0 mg/day” (EMA, 2009b). The content of  $\alpha$ - and  $\beta$ -thujone in *Artemisia vulgaris* L. is normally below the levels found in *Artemisia absinthium* L., but the concentration is variable (Pelkonen, Abass, and Wiesner, 2013). The essential oil from the herbal stem and flowers of *Artemisia vulgaris* L. contain approximately 56.3%  $\alpha$ -thujone and 7.5%  $\beta$ -thujone (EFSA, 2012) but, because of their low solubility in water, it is difficult to predict the quantity extracted by traditional infusion.

$\alpha$ -thujone and  $\beta$ -thujone are both responsible for neurotoxic effects,  $\alpha$ -thujone being 3-4 times as potent as  $\beta$ -thujone (Höld, Sirisoma, and Casida, 2001). The neurotoxicity is due to the rapid action of thujone in modulating the GABA-gated chloride channels and accounts for the epileptiform convulsions that are usually present in cases of acute poisoning (EMA, 2012). Convulsions are normally preceded by other less specific symptoms, such as vasodilation leading to hypotension, tachycardia and respiratory problems (IPCS, 1981).

Some case reports of severe intoxication due to the consumption of herbal preparations containing thujone have been published. Blindness, hallucinations and epileptiform convulsions sometimes progressing to unconsciousness are the most usual clinical patterns described (Burkhard, Brukhardt, Haenggeli, and Landis, 1999; Holstege, Baylor, and Rusyniak, 2002; Lachenmeier, Walch, Padosch, and Kröner, 2006; Strang, Arnold, and Peters, 1999). Acute poisoning due to thujone is most frequently associated with the consumption of *Artemisia absinthium* L. and alcoholic beverages containing its essential oil. Like other famous artists, Vincent Van Gogh suffered from absinthism, which differs from alcoholism in presenting episodes of delirium and epilepsy (Arnold, 1988; Holstege et al. 2002).

The acute oral toxicity (LD<sub>50</sub>) of thujone in laboratory animals (mouse, rat, guinea pig) has been reported at doses between 192 and 500 mg/kg body weight (EMA, 2012; SCF, 2002). In a study performed in rats, where thujone was administered by gavage on five days a week for 13 weeks, a NOEL (no-observed effect level) of 12.5 mg/kg bw for convulsion was established in males (Surber, 1962). In a similar study, where thujone was administered by gavage 6 times/week for 14 weeks, the NOEL for convulsive effect was 10 mg/kg bw in males and 5 mg/kg bw in females (Margaria, 1963). In 2-year studies performed by the National Toxicology Program (NTP, 2011) a NOEL for mortality of 12.5 mg/kg bw was identified in rats (although clonic seizures were observed at this level - the lowest administered), while in mice a NOEL for both mortality and seizure was established at 12 mg/kg bw.

Few data on the pharmacokinetic and toxicokinetic of thujone in humans are available. Max (1990) reported that a dose of 2-4 mg of thujone (0.03-0.06 mg/kg body weight), consumed with an alcoholic drink, did not induce the acute effects described in the scientific literature.

Hinkelbein (2004) confirmed that a dose of 3.5 mg of thujone, corresponding to 0.05 mg/kg bw, should be safe.

In 2012, EMA published a statement on the use of herbs containing thujone with the evaluation of acute and chronic toxicity of thujone in humans (EMA, 2012). The conclusions were that animal studies can be considered significant in calculating the human sensitivity to thujone, even though a direct extrapolation of the dose responsible for acute poisoning is uncertain. According to the available data, daily doses of 1.5-3.85 mg would not produce neurological disorders, while doses of 15 mg could affect attention and mood. In agreement, Dettling, Grass, Schuff, Skopp, Strohbeck-Kuehner, and Haffner (2004) showed that the intake of 17-20 mg in a person of 70 kg bw (0.24-0.28 mg/kg bw day) could be responsible for mild effects, such as problems in driving or operating machinery. On the basis of these results, EMA indicated 3 mg as the maximum safe daily dose for humans (EMA, 2009a).

The study reported in this paper describes a case of poisoning, which occurred in a 49-year-old man presenting a manic state after the ingestion of 1 L of *Artemisia vulgaris* infusion. The specific clinical symptoms suggested poisoning by thujone and this was confirmed by its presence in the patient's serum and urine. Two analytical approaches were used: 1) a newly developed and validated HPLC-FLD method, and 2) a published method based on gas chromatography coupled with a flame ionization detector (FID).

### **Case report**

A 49-year-old man was admitted to the University Hospital of Zurich in a manic state. According to the patient, he was habitually drank 1 L/day of an infusion prepared from *Artemisia vulgaris*. There were no details on how the infusion was prepared, but he had continued the practice for about three years.

Blood samples were taken on the day of admission (day 0) and on the following day (day 1), while urine was sampled only on day 1. Approval from an ethics committee or institutional review board was not necessary for the analyses performed, since they had been

requested to establish the source of the poisoning. The patient gave Informed consent for publication of this case.

## **Materials and methods**

### ***Purified standards***

The purified standard of  $\alpha,\beta$ -thujone (80% purity; 70%  $\alpha$ -thujone and 10%  $\beta$ -thujone) was from Sigma Aldrich (Steinheim, Germany). This mixture was the product with the highest quality available in the short time necessitated by the patient's condition and its standard of purity was considered sufficient for our purposes.

### ***Reagents***

The reagents (LC grade) were: acetone (Farmitalia Carlo Erba, Milano, Italy); L-ascorbic acid (Sigma-Aldrich, Steinheim, Germany); acetonitrile, methanol and water (VWR International, Fontenay-sous-Bois, France).

## **HPLC-FLD METHOD**

### ***Preparation of standard solutions and calibration curve***

The reference standard solution contained 0.724 g/mL of  $\alpha$  and  $\beta$ -thujone (taking into consideration purity of 80.0% and density 0.925 g/mL). An aliquot of 10  $\mu$ L of the standard solution was diluted in 10 mL of methanol to a final concentration of 0.724 mg/mL. Standard solutions were added to a healthy volunteer's serum ("control" sample) to concentrations of 14.5, 29.0, 57.9 and 72.4  $\mu$ g/mL of thujone.

### ***Preparation of biological samples***

Aliquots of 700  $\mu$ L of the patient's and control serum (the latter without and with the addition of purified thujone) were added to 20  $\mu$ L of an aqueous solution of L-ascorbic acid (1% w/v), used as a preservative; 700  $\mu$ L of acetone was added to each and the resulting solutions were thoroughly vortexed and centrifuged at 2500 r.c.f. for 5 minutes (Hermle Labortechnik, Wehingen, Germany). The supernatants were filtered through a 0.45  $\mu$ m syringe filter (VWR International, Fontenay-sous-Bois, France) and injected into the HPLC.

The urine sample was filtered as such on a 0.45 µm filter and injected into the chromatographic equipment.

#### ***Chromatographic conditions***

For the identification and quantification of thujone in the patient's serum, a specific HPLC method combined with fluorimetric detection was developed. This method proved incapable of separating the two isomers, but this was achieved by gas chromatography, see below. The equipment consisted of an Intelligent PU-880 pump (Jasco, Tokyo, Japan), a fluorescence detector FP-1520 (Jasco, Tokyo, Japan), a sample injection valve Rheodyne 7725 with 20 µL loop (Cotati, California, USA) and a column LiChrospher® 100, RP-8, 250 x 4 mm, particle size 5 µm (Merck KGaA, Darmstadt, Germany) heated at 30 °C. ChromNAV software (Jasco, Tokyo, Japan) was used for data integration. The analysis was performed by isocratic elution at a flow rate of 1 mL/min with a mobile phase containing acetonitrile:water 55:45 (v/v). The fluorescence detector was set at 220/290 nm ( $\lambda_{ex}/\lambda_{em}$ ).

#### ***Method validation***

The HPLC method was validated according to internationally recognized guidelines for analytical methods (FDA, 2013; Peters, Drummer, and Musshoff, 2007; Shabir, 2003). The following parameters were calculated: system suitability test (SST), linearity, sensitivity as LOD (Limit of Detection) and LOQ (Limit of Quantitation), selectivity, accuracy and precision.

#### ***GC-FID***

##### ***Chromatographic conditions***

Gas-chromatographic analyses were performed according to Dybowski and Dawidowicz (2016) with some modifications. The equipment included a DANI 8610 gas-chromatograph (DANI Instruments, Cologno Monzese, Italy) with a flame ionization detector (DANI 86/10, DANI Instruments, Cologno Monzese, Italy). For chromatographic separation of  $\alpha$ - and  $\beta$ -thujone and an internal standard, naphthalene, a Supelco SLB®-5ms fused silica capillary column (length 30 m, i.d. 0.25 mm,  $d_f$  0.25 µm) was used (Sigma-Aldrich, Steinheim, Germany). Helium was used as a carrier. The injector temperature was set at 290 °C. The oven temperature program employed was: 1 min at 50 °C followed by an increase of 6 °C/min up to 110 °C and then at a rate of 20 °C/min up to 280 °C.

##### ***Preparation of standard solutions and calibration curve***

A calibration curve was prepared by injecting solutions of purified  $\alpha$ - and  $\beta$ -thujone in the presence of naphthalene as an internal standard (ISTD) (Sigma-Aldrich, Steinheim, Germany). Standard solutions were prepared by diluting the commercial standard in dichloromethane. The final concentrations were: for  $\alpha$ -thujone 109, 52, 42, 18, 1  $\mu\text{g/mL}$ ; and for  $\beta$ -thujone 20, 10, 8.3, 0.2  $\mu\text{g/mL}$ . Internal standard concentration was 25  $\mu\text{g/mL}$  in all the samples. Two microliters of the sample were injected into the GC at least three times.

#### **Sample preparation**

Sample preparation was modified from the original method, since the biological fluids most usually received by emergency rooms are serum (not plasma) and urine. Dichloromethane (200  $\mu\text{L}$ ), containing 25  $\mu\text{g/mL}$  of ISTD, was added to 200  $\mu\text{L}$  serum and the two phases were subjected to vortex mixing for one minute. The resultant emulsion was centrifuged at 2500 r.c.f. for 5 minutes. The upper aqueous phase was then separated and extracted again with 200  $\mu\text{L}$  dichloromethane containing ISTD. The organic layers were combined and analyzed.

### **RESULTS**

The patient arrived at the emergency room in a manic state. He reported a habit of drinking (daily and in large quantity) an infusion of *Artemisia vulgaris*. The clinical symptoms presented by the patient were compatible with an exposure to thujone, a neurotoxic molecule contained in variable quantity in *Artemisia* spp, but normally hardly present in *Artemisia vulgaris* (Abad et al. 2012).

To test the hypothesis that thujone was responsible for the observed poisoning, thujone was quantified in the patient's serum and urine by HPLC-FLD and by gas chromatography coupled with FID.

#### **HPLC-FD METHOD**

##### **System suitability test (SST)**

In this test the following parameters were calculated: retention factor (K), separation factor between two neighboring peaks ( $\alpha$ ), peak tailing factor and column efficiency (number of theoretical plates). The analysis was performed five times. **Table 1** shows the results of the SST obtained from a control serum spiked with standard thujone at a concentration of 54.3



µg/mL. The chromatographic system proved to be efficient and suitable for the quantification of thujone, with retention factor ( $K$ )  $\geq 2$ , separation factor ( $\alpha$ )  $> 1$ , and symmetry factor (SF)  $\leq 2$ . However, the method does not separate the two isomers of thujone, unlike gas chromatography, see below.

### **Linearity**

Standard solutions (0.724 mg/mL) were added to a “blank” serum to the final concentration of 14.5, 29.0, 58.0 and 72.4 µg/mL and were prepared as previously described; each solution was analyzed at least three times.

A linear regression was obtained by plotting the areas of analyte peaks vs. the nominal concentrations. The method was linear between 14.5 and 72.4 µg/mL, with a correlation coefficient ( $R^2$ ) of  $>0.99$ . The linear regression equation was  $y = 253\,137x - 189\,570$ , where  $y$  refers to the peak area and  $x$  refers to concentration.

### **Sensitivity (LOD and LOQ)**

The limit of detection (LOD) and the limit of quantitation (LOQ) were determined by spiking control serum with decreasing concentrations of thujone standard solutions: LOD was established at a signal-to-noise ratio of 3, while LOQ at a signal-to-noise ratio of 10.

Furthermore, the precision at LOQ was evaluated with five independent injections.

The LOD and LOQ calculated in spiked serum calibration samples were 1.36 µg/mL and 4.53 µg/mL, respectively. The precision at LOQ was 19.4%, below the acceptable value of 20%. All values reported above were suitable for an accurate determination of thujone in serum samples.

### **Selectivity**

The possible interaction between analytes and endogenous matrix (serum) compounds was investigated by adding standard thujone to a control serum at the final concentration of 54.3 µg/mL. Selectivity requires that the peak area of compounds eluting together with the analyte of interest is less than 20% of the peak area of sample at LOQ. As shown in **Figure 2**, thujone is clearly identified, since no significant peak was present at its retention time. The two isomers are not separated by HPLC; they are shown to have slightly different retention times in gas chromatography, as shown below.

### ***Accuracy and precision***

Accuracy and precision were determined by spiking control serum with two quantities of purified thujone, at final concentrations of 18.1 and 54.3  $\mu\text{g/mL}$ . Accuracy describes the closeness of a measurement to the true value and is calculated as the percentage ratio between the experimentally measured values and the nominal ones. Precision was determined by calculating the variation coefficient (RSD%) of the peak areas of five replicates injected in the same day. The method was precise and accurate, since RSD% was below 15% (5.1-5.7%) and the calculated accuracy was always within  $\pm 15\%$  (106.8 to 108.5) of the nominal concentration.

### ***Recovery***

To evaluate the recovery in biological fluids, control serum was spiked with a standard solution at the final concentration of 25  $\mu\text{g/mL}$ . Recovery was  $94.2 \pm 10.3\%$ .

### ***Quantification of thujone in patient's serum***

The chromatograms of the patient's serum at day 0 and day 1 are illustrated in **Figure 3**. Using the regression line prepared in serum, thujone concentrations were determined and corresponded to  $27.7 \pm 3.5$  and  $24.1 \pm 0.15$   $\mu\text{g/mL}$  (mean  $\pm$  SD), at day 0 and 1, respectively. The identification of thujone was confirmed by spiking the serum with a solution of purified standard (**Figure 4**).

### ***Quantification of thujone in patient's urine***

The method was similarly validated for the analysis of urine, by using a linear regression obtained by spiking control urine with known quantities of purified standard. **Figure 5** shows the chromatograms of a control urine without and with addition of a standard solution of thujone at a final concentration of 72.4  $\mu\text{g/mL}$ . No interfering peaks were present at the retention time of thujone in the control sample. No peak at the retention time of thujone was detectable ( $< \text{LOD}$ ) in the patient's urine (not shown).

### **GC-FID METHOD**

To confirm unambiguously the presence of thujone in the patient's biological fluids, the method of Dybowski and Dawidowicz (2016) was also applied. An example of the gas-chromatographic separation of the two stereoisomers of thujone and the internal standard naphthalene is illustrated in **Figure 6**.

A calibration curve was obtained plotting the ratio between the area of the analyte and that of the internal standard (ISTD, naphthalene) vs. the respective concentrations. Within the concentrations used, the linearity was highly satisfactory, having  $R^2 > 0.999$  for both thujone isomers.

The limit of detection (LOD) was calculated at a signal-to-noise ratio of 3 and LOQ at a signal-to-noise ratio of 10. The latter was obtained by comparing the area of thujone signals and that of six peaks (average) of baseline noise from four injections of extracts (dichloromethane) of control serum samples spiked with a standard solution of thujone. LOD and LOQ were 0.11  $\mu\text{g/mL}$  and 0.37  $\mu\text{g/mL}$ , respectively, for  $\alpha$ -thujone, and 0.10  $\mu\text{g/mL}$  and 0.34  $\mu\text{g/mL}$ , respectively, for  $\beta$ -thujone.

To evaluate the recovery in biological fluids, the control serum was spiked with standard thujone to a concentration of 25  $\mu\text{g/mL}$  ( $\alpha = 23.0 \mu\text{g/mL}$ ,  $\beta = 4.2 \mu\text{g/mL}$ ) and injected. The results showed an average value of  $20.5 \pm 1.1 \mu\text{g/mL}$  ( $\alpha = 15.5 \pm 0.9 \mu\text{g/mL}$ ,  $\beta = 2.8 \pm 0.16 \mu\text{g/mL}$ ). The recovery was acceptable, being close to 82% for both total thujone and separated stereoisomers.

**Figure 7** shows the gas-chromatogram of the patient's serum at day 0, when the total thujone concentration was:  $22.3 \pm 1.3 \mu\text{g/mL}$  ( $\alpha = 18.9 \pm 1.0 \mu\text{g/mL}$ ,  $\beta = 3.4 \pm 0.2 \mu\text{g/mL}$ ). In agreement with the HPLC method, no thujone could be detected in urine ( $< \text{LOD}$ ) (not shown).

## DISCUSSION

The case reported in this paper describes a patient who experienced a manic state after the consumption of an infusion of *Artemisia vulgaris*. The patient reported having consumed similar infusions for at least three years with no adverse effect. No neurotoxic effect related to *Artemisia vulgaris* derivatives could be found in the scientific literature, not surprisingly in view of the small amount of thujone in this species (Abad et al. 2012). Neurotoxic effects have been reported only for the essential oils of *Artemisia absinthium*, which contain significantly

higher amounts of thujone (Lachenmeier et al. 2006). On the other hand, the symptoms described by the first-aid physicians attending this patient were identical with those associated with thujone intoxication by Lachenmeier et al. 2006 and Pelkonen et al. 2013). Although the presence of thujone in essential oils and in alcoholic extracts is well documented, aqueous extracts have been considered safe because of the low water solubility of this neurotoxic compound (Capasso, Grandolini, and Izzo, 2006).

To confirm the presence of thujone in this patient's serum and urine, two analytical approaches were used: 1) an HPLC-FLD method, set up and validated for this study; and 2) the gas-chromatographic method using a flame ionization detector (FID) published by Dybowski and Dawidowicz (2016).

For gas chromatography, the sample preparation was slightly modified from the original method since the biological fluids most usually obtained in the emergency room are serum and urine, and values measured in these fluids could be underestimated, because the fraction bound to plasma proteins would not be taken into account

Although slightly different in performances (HPLC gives superior recovery, but shows lower sensitivity), both methods were considered useful in the identification and quantification of thujone in the case reported. With both methods, the concentration of total thujone in serum at day 0 was close to 25 µg/mL confirming the intake of this molecule with the infusion. The differences in recovery could account for the small difference in serum concentrations obtained by the two methods (27.7 and 22.3 µg/mL, by HPLC and GC, respectively). With both methods, thujone was below LOD when measured in urine. Since the raw material (herbal mixture) and the infusion were not available for further analytical assessments, it is only possible to hypothesize the following:

- 1) An unusually high concentration of thujone in the *Artemisia vulgaris*. Even though there is no direct correlation between solubility in water and oil, a recent paper showed that the content of thujone in essential oil can vary significantly (Obistioiu et al. 2014).
- 2) The patient has a low metabolic efficiency, which increases the half-life of 24 hours determined by Lis-Balchin et al. (2006). The very similar levels of thujone in the patient's serum at day 0 and day 1 supports this hypothesis.

- 342 3) Thujone poisoning due to the chronic exposure (three years). Chronic toxicity of  
343 thujone has been described in rats and mice, which experienced clonic and tonic  
344 seizures and increased incidence of non-neoplastic lesions in brain, spleen, kidney  
345 and pituitary gland after two years of  $\alpha,\beta$ -thujone intake (NTP, 2011);  
346 4) Possible adulteration/contamination of the *Artemisia vulgaris* with other species, e.g.  
347 *Artemisia absinthium*.

348 To our knowledge, this is the first study where thujone was measured in human serum after a  
349 case of poisoning. Other quantifications in human serum have been reported in studies  
350 performed on volunteers (Kröner, Padosch, Lachenmeier, and Madea, 2005; Dybowski and  
351 Dawidowicz 2016).  
352 Kröner et al. (2005) described a pilot study in which two subjects consumed 110 mL of  
353 absinthe containing 3.85 mg thujone within 15 min; thujone was undetectable in their blood  
354 (LOD: 0.34 ng/mL). Dybowski and Dawidowicz (2016) detected thujone in plasma from five  
355 volunteers one hour after the consumption of an alcoholic solution containing approximately  
356 300  $\mu$ g of  $\alpha,\beta$ -thujone. Plasma values ranged from 22.3 to 37.6 ng/g. Comparing the values  
357 found in our patient's serum ( $\mu$ g/mL) with those measured in volunteers' serum (ng/mL), it is  
358 evident that our patient had been exposed to very high doses of thujone, during his long-term  
359 intake (daily for three years) with chronic accumulation of thujone, which is lipophilic.

## 361 CONCLUSION

362 This paper describes the first case of poisoning due to *Artemisia vulgaris*, in which thujone  
363 was identified as a biomarker of toxicity caused by prolonged excessive exposure. The case  
364 points out some general critical issues related to the consumption of botanicals. The  
365 increased use of botanicals/herbs in recent years is not always associated with suitable  
366 quality and safety control. There is a general belief by consumers that "natural" is always safe,  
367 but adverse effects of botanicals are far from rare.

368 The case also allowed the comparison of two different analytical approaches to measure  
369 thujone in serum and urine. The method developed for this study (HPLC-FLD) proved to be  
370 simple, relatively cheap and sensitive enough to measure small quantities of thujone, as in a  
371 case of poisoning.

372

### 373 **CONFLICTS OF INTEREST**

374 The authors declare no potential conflicts of interest with respect to the authorship and/or  
375 publication of this article.

376

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381 *views or future policy in these areas.*

382

### 383 **Author Contributions**

384 Chiara Di Lorenzo designed the study; Francesco Ferretti and Gianfranco Frigerio produced  
385 different parts of the analytical data; Enzo Moro collected test data; Alessandro Ceschi and  
386 Francesca Colombo collected and interpreted the results; Saskia Lude revised the work;  
387 Patrizia Restani drafted and reviewed the work.

388

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499 **Table 1** - System suitability test for thujone (n = 5)

<b>t<sub>R</sub><sup>a</sup> (min)</b> <b>(mean ± SD)</b>	<b>K<sup>b</sup></b> <b>(mean ± SD)</b>	<b>α<sup>c</sup></b> <b>(mean ± SD)</b>	<b>SF<sup>d</sup></b> <b>(mean ± SD)</b>	<b>N<sup>e</sup></b> <b>(mean ± SD)</b>
18.22 ± 0.05	12.84 ± 2.54	1.29 ± 0.01	0.97 ± 0.02	10316 ± 405

500 **LEGEND:**

501 <sup>a</sup>t<sub>R</sub> = Retention time; <sup>b</sup>K (Retention factor) = (t<sub>R</sub> - t<sub>0</sub>)/t<sub>0</sub>, where t<sub>R</sub> and t<sub>0</sub> are retention times  
502 of thujone and solvent, respectively; <sup>c</sup>α (Separation factor) = (t<sub>R</sub> - t<sub>0</sub>)/(t<sub>R1</sub> - t<sub>0</sub>), where t<sub>R</sub>  
503 and t<sub>R1</sub> are retention times of thujone and a neighboring peak, respectively; <sup>d</sup>SF (symmetry  
504 factor) = W<sub>0.05</sub>/2f, where W<sub>0.05</sub> is width of the peak at 5% height and f the distance from the  
505 peak maximum to the leading edge of the peak, the distance being measured at a point 5% of  
506 the peak height from the baseline. <sup>e</sup>N (number of theoretical plates) = 16/(t<sub>R</sub>/W)<sup>2</sup>, where W is  
507 the peak width at its base.

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